Electrophysiological Responses from *Culicoides* (Diptera: Ceratopogonidae) to Stimulation with Carbon Dioxide

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ABSTRACT Because of their impact on human and veterinary health, there is considerable interest in understanding how *Culicoides* use olfactory cues in host location. The adequate chemical stimulus for sensilla located on the maxillary palps was determined for several species of female *Culicoides*. Electrophysiological studies identified and characterized the sensory neurons on *Culicoides* maxillary palps that responded to stimulation with low concentrations of CO_2. The concentration response function in different background concentrations of CO_2 was established for *C. furens* (Poey), *C. stellifer* (Coquillet) and *C. mississippiensis* Hoffman. Comparisons were made to previously studied CO_2-sensitive neurons in mosquitoes. Understanding what sensory signals the host releases and how they are detected may lead to the development of strategies aimed at controlling these insects.

KEY WORDS attractants, *Culicoides furens*, olfaction, electrophysiology, carbon dioxide

Culicoides IS THE MOST important genus of Ceratopogonidae with respect to the health and comfort of humans and animals (Kettle 1965, Linley et al. 1983). Annoyance from adult female biting midges often may become serious enough to curtail outdoor recreational and work-related activities. Their presence also has an adverse effect on tourism and land development, especially in coastal areas (Linley and Davies 1971). Animal and human health are affected by disease organisms, transmitted to them by adult females, which include protozoans, filarial worms and viruses (Kettle 1965, Blanton and Wirth 1979, Linley et al. 1983).

Control of adult biting midges has met with limited success. Traditional ground and adulticide sprays provide little long-term relief (Linley 1976), stimulating the development of novel control methods. One management concept under consideration is the use of semiochemical-baited traps/targets to capture/kill nuisance adult biting midges. The success of this approach depends upon the development of efficient trapping technology, the discovery of effective attractants and subsequently, strategic placement of these baited traps/targets for maximum impact on the target population.

The objective of the work reported herein was to use electrophysiological methods to identify and characterize the sensory neurons on *Culicoides* maxillary palps that respond to stimulation with carbon dioxide. CO_2, emitted by a potential host, is used by adult female *Culicoides* to help locate that host (Nelson 1965). Other chemicals released by the host also may be involved and such chemical signals presumably are detected by receptor neurons in sensilla on the antennae or mouthparts. We were interested in determining chemical stimuli that effectively stimulate sensory structures located on the maxillary palps of female *Culicoides*. Although considerable research has focused on the morphology (Braverman and Hulley 1979, Chu-Wang et al. 1995, Wirth and Navai 1978, Jobling 1928, Jamnback 1965, Kline and Axtell 1999), ecology (Blackwell et al. 1992, 1994, Kettle 1972, Lardeux and Ottenwaelder 1997, Fallis and Bennett 1961), host preference (Fallis and Bennett 1961), trapping (Holbrook and Bobian 1989, Blackwell et al. 1992) and taxonomy (Holbrook et al. 2000, Tabachnick 1996, Velten and Mullens 1997, Holbrook and Tabachnick 1995) of *Culicoides spp.*, relatively little electrophysiological research has been conducted to determine the physiological characteristics of the sensory system. Electroantennogram (EAG) studies on *C. impunctatus* Goetghebuer demonstrated responses from antenna stimulation with 1-octen-3-ol (Blackwell et al. 1996, Blackwell et al. 1997). In addition, EAGs recorded stimulation with several host plant repellents including methyl salicylate and isothiocyanate (allyl, butyl, phenyl, and 2-phenylethyl) (Blackwell et al. 1997). However, physiological recordings from maxillary palp structures are lacking, as are single unit studies of neuronal specificity.

In many species, such as *C. furens* (Poey), small clusters of 10–15 sensilla are located in depressions along the distal edge of the third subsegment of the

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maxillary palp. In other species, such as *C. melleus* (Coquillett), the sensilla are not restricted to these pits, but rather are uniformly distributed over the subsegment. Both sexes of *Culicoides* possess these basiconic sensilla that share general morphological similarity with sensilla found on the maxillary palps of mosquitoes. From previous work (Kellogg 1970, Grant et al. 1995, Grant and O'Connell 1996), we know that basiconic sensilla on the maxillary palps of mosquitoes contain three primary receptor neurons, one of which responds to carbon dioxide. To determine if a similar physiological-morphological relationship exists in ceratopogonids, we recorded single neuron responses from basiconic sensilla located on the third subsegment of the female's maxillary palps in several *Culicoides*. In the current report, we characterize the response properties of these sensory receptor neurons and compare these responses to those reported previously for *Aedes aegypti* (L.) (Grant and O'Connell 1996).

Materials and Methods

Insects. Female *C. furens* were collected as adults from traps located in Jamestown, RI; East Greenwich, RI; Cape Cod, MA; and Cedar Key, FL. *C. mississippiensis* Hoffman and *C. stellifer* (Coquillet) were collected from Cedar Key, FL. The majority of insects came from Cedar Key, FL. Because field collected insects were tested, the physiological state of the insects, with respect to age, egg production, or blood feeding, was unknown. Once in the laboratory, insects were kept at 78–80°F at 68–75% RH under a 12:12 L:D cycle with 10% sucrose-in-water available ad lib. Under these conditions, adult *Culicoides* spp. could be kept alive for at least 7 d.

Electrophysiological Methods. Electrophysiological techniques recorded extracellular responses from the receptor neurons located in the *sensilla basiconica* on the maxillary palps of *C. furens*, *C. mississippiensis*, and *C. stellifer*. Details of the recording methodology have been described in previous reports (O'Connell 1972, Grant and O'Connell 1986) and are summarized below.

Insect Preparation. To ensure stable recording, the insect was carefully immobilized with the maxillary palps positioned in a manner that allowed unobstructed access with the recording microelectrode to the targeted sensillum. Adults were immobilized on a 1-cm^2 glass plate mounted in a Plexiglas holder with minute strips of sticky tape (Double Stick Tape; Scotch, 3M) or fiber threads used to secure the thorax, abdomen, legs, and wings. The head and mouthparts were positioned on a transparent ledge with the sensilla on the maxillary palps facing the recording electrode. Once the insect was secured, the entire holder was positioned under the objectives of a compound light microscope, from which the microscope stage was removed. The microscope light source was adjusted to illuminate the palps from beneath. This method of *trans*-illumination allowed a clear view of the sensilla. The microscope was mounted on an X-Y translational stage, which allowed us to view different portions of the preparation without moving the insect holder. A standard compound microscope, with an effective magnification of ≈400×, has sufficient working distance to allow unimpeded access with the recording microelectrode.

Electrode Manufacture and Recording. The recording microelectrodes were hand made of straightened 125-μm diameter tungsten wire that was electrolytically sharpened to a tip diameter <1 μm (Hubel 1957, Galbreath and Galbreath 1977) in a 10% electrolyte solution. Adjustments in current flow and rate of withdrawal of the electrode during the sharpening process made it possible to control both the taper and the final tip diameter of the resulting microelectrode. These latter factors were optimized for each sensillum type, to minimize the force required for cuticle penetration.

Following manufacture, the microelectrode was mounted in a vibration damped glass tube that was held in low drift, high-gain (800×) Leitz micromanipulator (Leitz-Wetzlar, Germany), which permitted precise and controlled electrode placement. An indifferent electrode, of similar design, was inserted into the eye of the insect under low magnification (40×), and the recording electrode was inserted (under high magnification 400×) into the base of an individual sensillum on the palp. Following placement of the electrodes, the electrical signals obtained from the neurons within the sensillum were band-passed filtered (300–1000 Hz), amplified (1000×), and sent in parallel to an audio monitor and a computer for subsequent data acquisition, action potential discrimination and analysis, and storage. We used either a modified Grass P16 amplifier or a Grass P511 AC preamplifier (Astro-Med, Inc., West Warwick, RI). The components of the basic work station, including the microscope, manipulators, preparation stage, and preamplifier, were all mounted on a vibration isolation breadboard and housed in a solid wall, aluminum Faraday cage. The electrical circuits required for this apparatus were isolated and independently earthed through an exterior ground rod.

Data Acquisition and Action Potential Discrimination. Data acquisition and analysis were accomplished using a software program, Autospike, developed by Jan van der Pers (Syntech, Hilversum, The Netherlands). The program was designed to record and analyze electrophysiological information from insect chemosensilla. This program runs on a Pentium PC (Windows 95/98 platform) with an analog-to-digital/digital-to-analog (AD/DA) interface via an IDAC controller. Action potential discrimination and sorting are accomplished with Autospike, which can analyze and discriminate impulse activity from sensilla with multiple neural inputs. Electrophysiological preparations from *Culicoides* typically last for several hours with no noticeable change in response properties.

Stimulus Delivery and Control. Although the main thrust of this report focused on CO_2 as a stimulus, we also were interested in determining the specificity of the neurons' response to other chemical stimuli. To accomplish this, we tested several compounds that

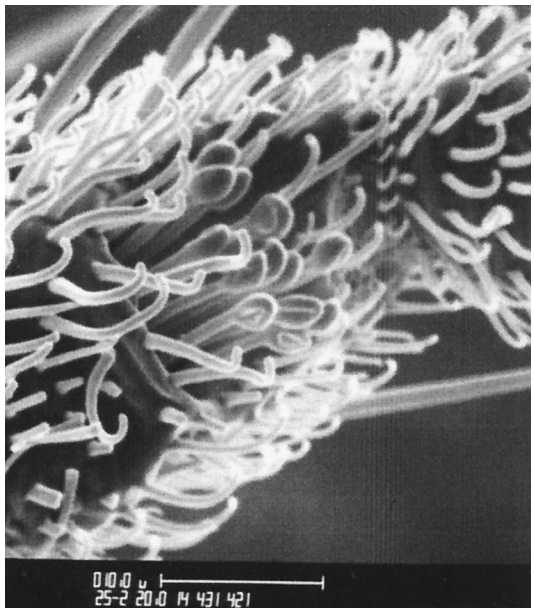

Fig. 1. SEM of a portion of the third segment of the maxillary palp from a female *C. furens*, showing the "club-shaped" *sensilla basiconica* in the ventral-lateral pit.

were effective electrophysiological stimuli for mosquito species. For this chemical specificity testing, compounds were obtained from the Center for Medical and Veterinary Entomology, USDA, ARS laboratories (Gainesville, FL) and diluted to desired concentration in mineral oil (Aldrich, Milwaukee, WI). One μl aliquots of the desired dilution were applied to filter paper strips (2 cm^2) positioned in glass cartridges. The cartridges were positioned at the end of the stimulus stream, immediately over the preparation.

For these experiments, two opposing gas streams were directed toward the exposed palp, one carrying the background (225 ml/min) and the other the stimulus (150 ml/min). With both streams on, the higher velocity background line prevented the stimulus stream from reaching the preparation. An independent controller activated two, fast-response (6 ms) 3-way Teflon valves (Parker-Hannifin Corp., Fairfield, NJ) and also initiated data collection by the software. The CO_2 stimuli were delivered from certified formulated gas cylinders (BOC Gas), each containing either 0, 150, 300, 600, 1000-ppm CO_2, 20% purified oxygen, and the remainder nitrogen. For CO_2 or chemical compounds, odor stimulation was accomplished by activating the stimulus line and deactivating the background line, thus allowing the chemical in the stimulus line to reach the preparation. Positioned immediately behind the preparation was a 4 inch-diameter tube that exhausts potential contaminants from the area immediately around the preparation.

To determine the effect of background concentration of the CO_2 response properties, the insects were allowed to equilibrate to the background CO_2 concentration for 2 min. Following this 2 min. period, a 2 s pulse of each of the concentrations (0, 150, 300, 600, 1000 ppm) was presented to the insect. Each pulse in this protocol was separated by 20 s. After all five concentrations were tested in a given background, the insect was exposed to a new background concentration of CO_2 and the 2 s protocol was repeated.

Statistical Analysis. Data transformed to log10 (n+1) for *C. furens* were analyzed with programs PROC analysis of variance (ANOVA) to evaluate the effects of preparation and treatment in a two-way ANOVA, and PROC MEANS/REGWQ for treatment mean comparisons with the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1985). Data were transformed and treatment means of *C. furens* and *Ae. aegypti* were compared for each of the five treatments with the PROC TTEST for two sample *t*-tests using the Satterthwaite procedure for unequal variances.

Scanning Electron Microscopy. The general morphology of the palps and the fine structure of the different sensilla of *C. furens* were characterized by scanning electron microscopy. Live specimens were fixed in osmium vapor for several hours and then adhered to a stub using tape and carbon paint. Specimens then were sputter-coated with gold: palladium (≈20 nm) and stored under vacuum until examination. Stubs were examined using an ETEC Autoscan microscope (Perkin-Elmer, Hayward, CA) at 20KV accelerating voltage and short working distances.

Results

Concentration Response of *C. furens* for CO_2. *Sensilla basiconica* on the maxillary palps (Fig. 1) of female *C. furens* contain a receptor neuron sensitive to stimulation with low levels of carbon dioxide. These neurons produced typical biphasic action potentials with peak-to-peak amplitudes of up to 350 μV. We recorded responses to stimulation with steps to 0, 150, 300, 600, and 1,000-ppm CO_2 (Fig. 2). These neurons were silent in background environments without CO_2 (Fig. 3). However, stimulation with concentrations as low as 150-ppm CO_2 reliably elicited action potential responses (12 of 15 preparations), indicating that the response threshold was between 0 and 150-ppm CO_2. With the exception of the responses to 600 and 1000-ppm CO_2, responses to stimulation with all other concentrations were statistically different from each other.

Neurons in the *C. furens* maxillary palp sensilla responded to rectangular pulses of CO_2 with a phasic-tonic pattern of discharge that was similar to that previously reported for mosquitoes (Grant et al. 1995, Grant and O'Connell 1996). In addition to the phasic response seen to a step increase in CO_2 concentration (Fig. 4), there was a similar phasic inhibition seen to a sharp decrease in CO_2 concentration (data not shown). Maxillary palp sensilla on female mosquitoes are distributed over the ventro-lateral surface of the fourth subsegment from the base. In contrast, *C. furens* maxillary palp sensilla were clustered together in a

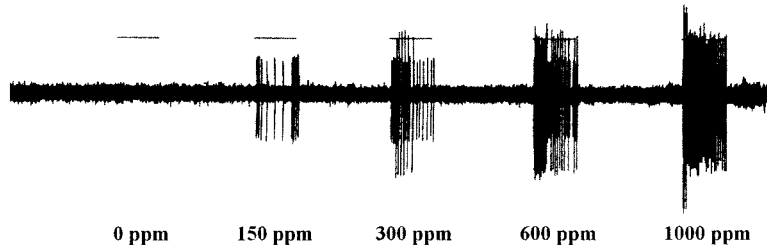

Fig. 2. Electrophysiological recordings from the maxillary palp sensilla of *C. furens* in response to stimulation with different concentrations of carbon dioxide. In this particular example, two amplitude action potentials are present. The neuron producing each set of action potentials responds to increasing concentrations of CO_2. The bars above the trace indicate the timing and duration of the 2-s stimulus pulse (0, 150, 300, 600, 1000 ppm CO_2). Between stimulations the preparation was held in a background environment of 0-ppm CO_2.

deep pit along the distal margin of the third subsegment. Due, in part, to the clustering of sensilla, occasional electrophysiological recordings produced multi-unit records (i.e., Fig. 2). Surprisingly, this occurred infrequently (4 of 23 preparations). On the occasions when it did occur, discrimination between the different amplitude action potentials was distinct enough so that both neurons could be studied. In the few cases where action potential discrimination was not reliable, the preparations were discarded and not used in analysis.

Temporal Pattern of Response. *Culicoides* maxillary palp sensilla were responsive to stimulation with CO_2, a compound associated with host-seeking behavior. However, processing in the central nervous system probably was dependent not only on the presence or absence of impulse activity, but also on the pattern of action potentials generated during the odor stimulus.

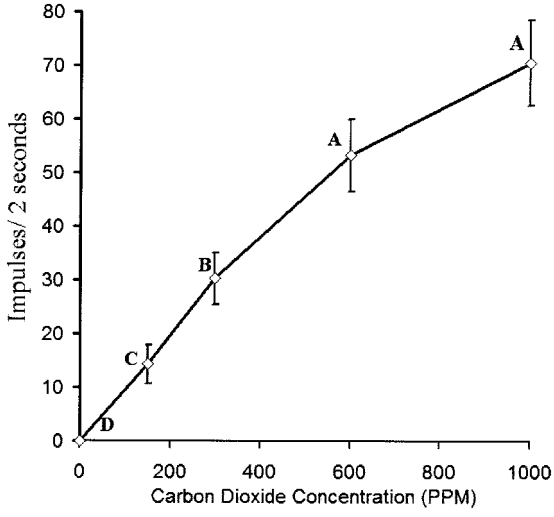

Fig. 3. Mean ± SEM carbon dioxide concentration response function for female *C. furens* [0, 150, 300, 600, 1000 ppm (n,15)]. Different letters indicate significant difference <0.05.

To determine the temporal pattern of activity for the CO_2-sensitive neurons in *C. furens* maxillary palp sensilla, we calculated the instantaneous frequency of action potentials generated by these neurons during the stimulus pulse (Fig. 4). As with mosquito CO_2-sensitive neurons (Grant et al. 1995, Grant and O'Connell 1996), *C. furens* CO_2-sensitive cells also responded with a sharp "phasic" burst of activity, lasting ≈100 ms, followed by a tonic response whose duration was dependent on the length of the stimulus pulse.

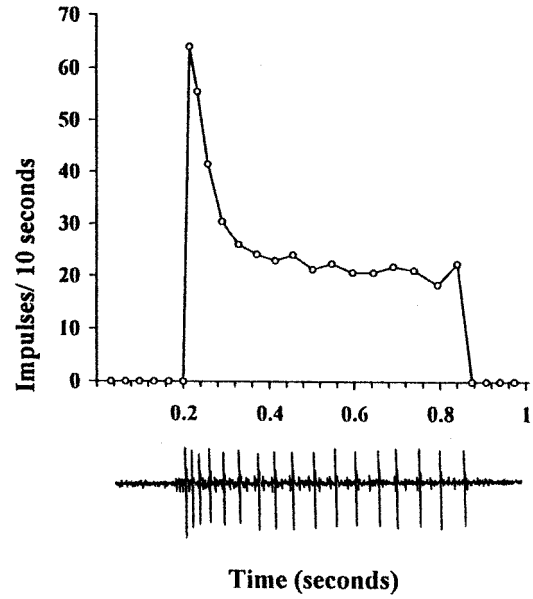

Fig. 4. The instantaneous frequency of action potentials generated by CO_2-sensitive neurons during the stimulus pulse. *Culicoides* CO_2-sensitive cells respond with a sharp "phasic" burst of activity, lasting ≈100 ms, followed by a tonic response whose duration is dependent on the duration of the stimulus pulse. In this experiment, the background concentration was 0 ppm and the stimulus concentration was 600 ppm CO_2.

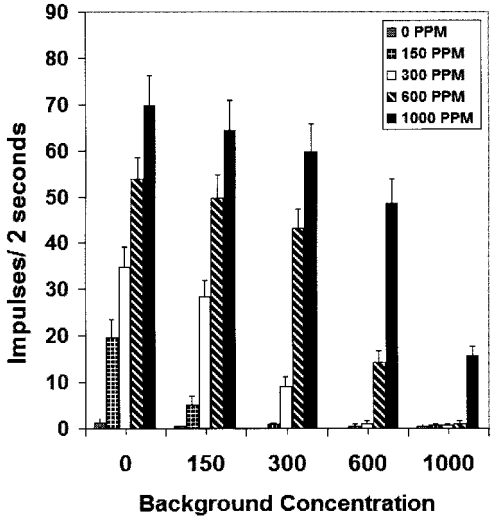

Fig. 5. Responses to CO_2 stimulation in different background concentrations of CO_2. The above set of histograms illustrates the response to stimulation with different concentrations of CO_2 (0, 150, 300, 600, 1000 ppm), in different background concentrations (0, 150, 300, 600, 1000 ppm). Each histograms represents the mean + SEM of 11 neurons including responses from *C. furens* (n,4), *C. mississippiensis* (n,2), and *C. stellifer* (n,5).

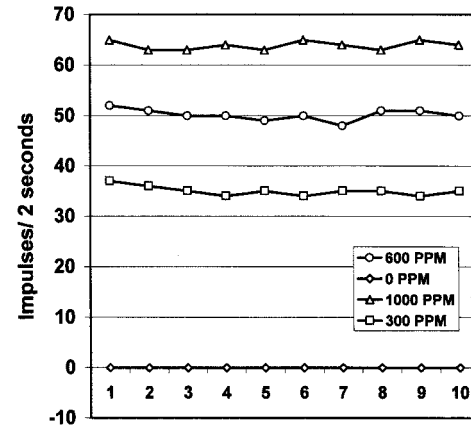

Fig. 6. Responses from a single CO_2-sensitive neuron on the maxillary palps of *C. furens* to repeated stimulation of 2-s pulses of different concentrations of CO_2. Pulses were separated by 8 s.

Responses in Different Background Concentrations of Carbon Dioxide. Figure 3 illustrates the neural response to step increases in CO_2 concentration in a background environment without CO_2 (0-ppm CO_2). The response magnitude is a relatively linear function of CO_2 concentration. However, biting insects in nature never encounter environments without CO_2. Therefore, we tested the same set of CO_2 concentrations used in Fig. 3 under different background levels of CO_2 (Fig. 5). For this analysis, we combined responses from *C. furens*, *C. stellifer*, and *C. mississippiensis*. The same basic pattern of response to stimulation in different background concentrations of CO_2 was seen with *Culicoides* (Fig. 5), as was previously reported for mosquitoes (Grant et al. 1995). Different amounts of adaptation were observed. The average numbers of impulses generated in different background concentrations, during a 2-s stimulus containing a given CO_2 concentration, were similar or slightly less as long as the background concentration was lower than the stimulus concentration. However, if background concentration was higher than the stimulus concentration, the average number of impulses generated to the stimulus step was greatly diminished. For example, in backgrounds containing 0, 150, or 300-ppm CO_2, the magnitudes of responses elicited by a stimulus containing 600-ppm CO_2 were comparable. However, in a background concentration of 1000-ppm CO_2, the magnitude of response to a similar stimulus containing 600-ppm CO_2 was greatly reduced.

Response to Repeated Stimulation by Carbon Dioxide. We tested the ability of an olfactory neuron to respond to repeated stimulation by CO_2 (Fig. 6). At all concentrations, little or no sensory adaptation was observed when intervals between pulses were at least 8 s. These data indicated that the response to CO_2 stimulation does not vary considerably over time. These two characteristics, the lack of sensory desensitization coupled with the stability of the response, may indicate that the insect is capable of following pulses of CO_2 to the source.

Responses from Different *Culicoides* spp. Females of other species of *Culicoides* also possess sensilla that are similar in morphology to the CO_2-sensitive structures found on *C. furens* (Kline and Axtell 1999). To test whether or not similar structures on different species also possess neurons sensitive to stimulation with CO_2, we recorded responses from *C. stellifer* and *C. mississippiensis* (Fig. 7). All three *Culicoides* exhibited comparable concentration-response functions with similar response thresholds and slopes. The slight differences seen among the three response functions may simply reflect the relatively small sample size *C. stellifer* ($n = 6$) and *C. mississippiensis* ($n = 3$). All three species have the same general morphological arrangement of basiconic sensilla, located in clusters along the swollen third sub-segment of the maxillary palp (Chu-Wang et al. 1995). This finding is of interest, because the three species differ in their ecology. *C. furens* and *C. mississippiensis* are both salt-marsh species, whereas *C. stellifer* is associated with fresh water environments.

Response to Other Olfactory Stimulation. Ultrastructural studies on the maxillary palp sensilla of *Culicoides* indicated that some sensilla were multiinnervated. This raised the possibility that like mosquitoes, the maxillary palp sensilla of *Culicoides* may also contain a neuron sensitive to compounds other that CO_2. To determine if *Culicoides* maxillary palps also possessed a neuron sensitive to other host attractants, we tested the sensitivity of the maxillary palp sensilla to several compounds found to be effective stimuli for mosquito maxillary palp neurons (Fig. 8).

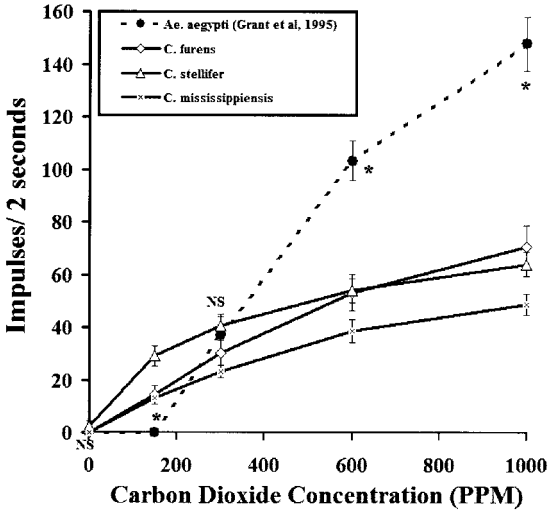

Fig. 7. Concentration response function to stimulation with five concentrations of carbon dioxide for three species of *Culicoides* [*C. furens* (n,15), *C. mississippiensis* (n,3), and *C. stellifer* (n,6)]. For comparison, I have included the previously published concentration response function for *Ae. aegypti* (n,13) (Grant et al. 1995). NS = no significant different; * <0.0001.

Consistent responses from any of the maxillary palp sensilla to any of the compounds were not observed, indicating that maxillary palp sensilla do not provide sensory input for these compounds.

Discussion

S. basiconica on the maxillary palps of *Culicoides* contain a receptor neuron sensitive to stimulation by concentrations of carbon dioxide. The *Culicoides* CO_2-sensitive neuron shares basic physiological characteristics with the mosquito CO_2 cell (Grant et al. 1995). Both respond to sharp increases and decreases in CO_2 with enhanced onset and offset response frequencies. Both are spontaneously active at or near ambient levels and both are silent in environments of 0-ppm carbon dioxide. However, there do appear to be two significant differences between *Culicoides* and *Ae. aegypti* response properties. First, in most cases (12 of 15 neurons), stimulation with 150 ppm CO_2 elicited a response in *C. furens*, which is statistically different from the response to stimulation with 0-ppm CO_2. However, in *Ae. aegypti*, higher concentrations were needed to elicit responses (at 150 ppm CO_2, only one of 13 responded) (Grant et al. 1995), indicating that the threshold of detection was lower for *C. furens* and consequently their neurons were more likely to be active at ambient levels. Second, the slopes of the concentration response functions were different. *Ae. aegypti* response function was steeper than *Culicoides*, indicating that a small change in stimulus concentration elicited a larger change in impulse activity indicating that *Aedes* were able to perceive smaller changes in carbon dioxide concentration than *Culicoides*. Although the slopes of the concentration functions were different, they overlapped near ambient levels (350 ppm CO_2).

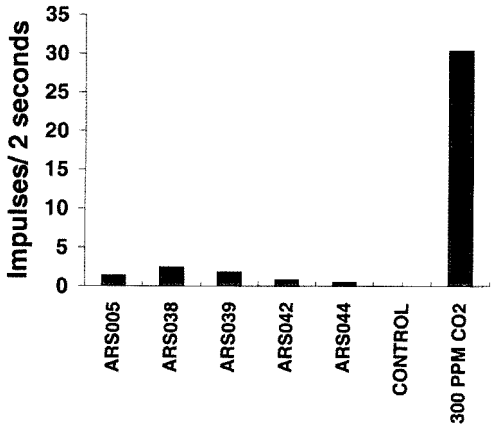

Fig. 8. Responses from maxillary palp sensilla to potential olfactory attractants. All responses are from *C. furens* (n,4–6 neurons). ARS005 = 1-octen-3-ol; ARS038 and ARS039 = 7 carbon ketones; ARS042 = 8 carbon, branched ketone and ARS044 = 6 carbon, branched ketone. All compounds were tested at a dose of 100 μg in mineral oil on filter paper, except 1-octen-3-ol that was tested at one or 10 μg dose. The control stimulus consisted of 1 μl of mineral oil on filter paper.

The significance of low threshold sensitivity to CO_2 in *Culicoides* is unclear. Ambient atmospheric levels of carbon dioxide are ≈350 ppm. So, it seems unlikely that having an ability to detect lower CO_2 concentrations than ambient would enhance the insect's host-seeking ability. Of course, many nematoceran insects are autogenous and require sugar feeding for survival (Stewart and Kline 1999). The environment contains many sources and sinks for CO_2. Actively photosynthesizing plants may produce local concentrations of carbon dioxide below ambient levels. It is possible that these insects are using CO_2 sensitivity at levels below ambient to locate portions of a plant that contains nectar for sugar feeding. *C. mississippiensis* is known to sugar feed from Yaupon Holly, *Ilex vomitoria* Aiton during the day and possibly during the night (Stewart and Kline 1999). The possibility exists that low concentrations of CO_2 in conjunction with other volatiles may serve as a plant attractant for sugar feeding. Whereas, higher concentrations of CO_2 may serve as a host-seeking attractant for blood feeding.

Sensitivity to low concentrations of CO_2 also may have no behavioral significance. Stange (1997) and Stange and Wong (1993) suggested that certain species of moths, which also have CO_2-sensitive neurons, actually are adapted to lower, preindustrialization levels of carbon dioxide. Although our methods differed from those of Stange in characterizing CO_2 sensitivity, perhaps the sensitivity to lower concentrations of CO_2 in *Culicoides* simply reflects an earlier sensitivity, which has not genetically adapted to the dramatic CO_2 increase caused by accelerated human-based emissions of carbon dioxide.

A second difference between an *Ae. aegypti* and *Culicoides* CO_2 concentration response function is the slopes of the curves. The mosquito function has a much steeper slope as compared with any of the functions observed for the three species of *Culicoides.* A steep slope indicates that small changes in concentration result in relative large changes in neural response. This indicates that the mosquito should have a peripheral sensory system capable of detecting smaller changes in CO_2 than *Culicoides*. Based on this characteristic of the mosquito, Grant et al. (1995) speculated that the ability to detect relatively small changes in CO_2 would mean that the mosquito could klinotactically follow a gradient of relatively small CO_2 increments to the source. This is in contrast to an alternative method of orientation, where detection of a stimulus induces the insect to "fly upwind." For *Culicoides*, where the slopes of the concentration-response functions are shallow, they presumably would not be able to detect such small gradients in CO_2 and, therefore, may not have the sensory system capable of allowing klinotaxic orientation. Of course, no one knows how a mosquito or a biting midge orients in a plume. However, these inherent differences in their physiological response functions to carbon dioxide indicate that these two groups of insects possess different peripheral sensory capabilities. These fundamental differences in the detection capabilities suggest that they may be using different physiological mechanisms to accomplish orientation and host seeking behavior.

Although the differences in the sensory systems between *Culicoides* and mosquitoes are interesting, it must be pointed out that the two sets of electrophysiological data were generated in different laboratories using slightly different methodology. For example, the rate of stimulus airflow between the two setups was different, raising the possibility that the stimulus could reach the preparation at slightly different times. Although such differences might be expected to produce some shift in reported threshold, it is harder to imagine how the slope of the function would be affected by these factors. Also, the mosquitoes were taken from laboratory colonies and their history with regard to blood feeding was known, whereas the *Culicoides* were collected in the field and nothing was known about their feeding history.

We have shown that neurons within the *Culicoides* maxillary palp sensilla are responsive to stimulation with CO_2, a compound associated with host-seeking behavior. Carbon dioxide has long been implicated (Rudolfs 1922) in activation or attraction of mosquitoes. However, basic features of this orientation behavior are still imperfectly understood. How the neural signal from the sensory system is processed by the central nervous system probably depends not only on the presence or absence of impulse activity, but also on the pattern of action potentials generated during the odor stimulus. To determine the temporal pattern of activity for the CO_2-sensitive neurons in *Culicoides* maxillary palp sensilla, we calculated the instantaneous frequency of action potentials generated by these neurons during the stimulus pulse. As with the mosquito CO_2-sensitive neurons (Grant et al. 1995, Grant and O'Connell 1996), *Culicoides* CO_2 cells also respond with a sharp "phasic" burst of activity lasting $\approx$100 ms, followed by a tonic response whose duration is dependent on the length of the stimulus pulse. This phasic onset to an increase in stimulus concentration may be important for orientation and navigation of the insect to the source. The phasic burst exaggerates concentration differences and thereby allows the insect to more accurately detect subtle changes in CO_2 concentration as it navigates in a CO_2 plume. Detection of small changes in concentration may be critical if the insect is actually klinotactically following an increasing CO_2 gradient to the host.

For many species of *Culicoides*, the addition of octenol to CO_2-baited traps enhances the number of insects collected (Kline et al. 1994). We know that in the maxillary palp sensilla of *Ae. aegypti*, there are two additional cells, one of which is sensitive to low doses of 1-octen-3-ol (Grant and O'Connell 1996). 1-octen-3-ol is a chemical attractant, which synergizes with CO_2 to greatly enhance trap catch for many species of mosquitoes and biting midges (Takken and Kline 1989, Kline 1994). We were curious to see if *Culicoides*, like the mosquito, also has other chemosensory neurons in the maxillary palp sensilla. To answer this question, we recorded responses from these sensilla to stimulation with other compounds that are effective stimuli for mosquitoes, including 1-octen-3-ol. None of the maxillary palp sensilla consistently responded to any of the compounds tested, other than carbon dioxide. In a limited number of recordings, where multiple impulse activity was seen, both neurons responded to CO_2 and neither responded to any other chemical stimuli tested, including 1-octen-3-ol. The lack of response to other olfactory stimuli from sensilla on the maxillary palps indicates that the sensory input required for the detection of these chemicals including octenol are probably found on other sensory structures of the insect, such as the antennae.

Many, if not all biting insects have evolved a complex sensory system designed to detect and locate vertebrate hosts for blood feeding, and it appears that odors play a role in this process. A ubiquitous cue released by all vertebrates, as a byproduct of respiration, is CO_2. We've begun to define the response properties of that portion of the peripheral sensory system of *Culicoides* that responds to CO_2. Knowledge about the peripheral sensory system is an important first step towards understanding the mechanisms of host seeking behavior of the biting insect. Understanding the similarities and differences in the physiology of *Culicoides*, as compared with other biting flies, should help explain the mechanisms used to elicit orientation and host seeking behavior. It is our belief that this knowledge of behavior will lead to the development of bio-rational methods for controlling these important pest species and ultimately reduce transmission of insect-vectored diseases.

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